of the Notch signaling pathway could induce S-phase entry, resulting in disc overgrowth. Notch acts in part by inhibiting the *Drosophila* retinoblastoma homolog Rbf, which normally functions to inhibit the S-phase-specific E2F transcription factor. Notch is also required for expression of Cyclin A; in addition, Notch may regulate a third, unknown factor to drive S-phase entry because coexpression of both E2F and Cyclin A did not rescue S phase in Notch mutant clones (Baonza and Freeman, 2005). Once cells transit through S phase into G2, they arrest until a further signal from differentiating neurons, dependent on the epidermal growth factor receptor, drives entry into mitosis (Baker and Yu, 2001).

What are the targets of Hedgehog and Dpp that mediate cell-cycle synchronization and arrest within the morphogenetic furrow? One likely target is String, the *Drosophila* homolog of the mitotic inducer Cdc25. String expression ahead of the furrow is required to drive cells through mitosis and into G1 (Heberlein et al., 1995; Mozer and Easwarachandran, 1999). Another candidate is Roughex, a gene required to inhibit Cyclin A-dependent kinase activity in the morphogenetic furrow. In the absence of Roughex, all cells in the furrow enter S phase prematurely. Roughex is expressed in the morphogenetic furrow, consistent with induction by Hedgehog, and the phenotype of *roughex* mutations is enhanced by mutations in *hedgehog* (Thomas et al., 1997). Direct cell-cycle targets of Dpp signaling have yet to be identified.

Other genes downstream of Hedgehog and Dpp likely include the G1 cyclin Cyclin D and the bHLH transcription factors Hairy, Atonal, and Daughterless, among others, whose expression is manifested as dramatic stripes across the disc. Hedgehog and Dpp also induce expression of the Notch ligand, Delta, and this expression is required for Notch activity in regulating S-phase entry behind the morphogenetic furrow (Baonza and Freeman, 2005). The integration of these complex signals results in the formation of regularly spaced groups of cells across the length of the furrow that are committed to differentiate into neurons. That cell-cycle entry behind the morphogenetic furrow depends on these same regulatory signals ensures that precursor cells will be generated at the right time and place and in the right numbers to pattern the adult eye. Although it is likely that the highly structured nature of the *Drosophila* compound eye requires an unusually stringent control, it is likely that proliferation and patterning will be linked by common regulatory signals in other organisms as well.

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Oxygen JNKies: Phosphatases Overdose on ROS

Proinflammatory cytokine TNFα triggers cell death by inducing reactive oxygen species (ROS). These then inflict cytotoxicity through downstream activation of the JNK MAPK cascade. Yet the mechanisms by which ROS trigger JNK signaling have remained elusive. In a recent issue of *Cell*, Kamata et al. now provide one such mechanism.

It might be surprising to know that we are not the first living beings to have caused a profound change in the composition of Earth's atmosphere. Over 2.2 billion years ago, oxygen became abundant as a result of "early" life, causing radical changes to the ecosystem with consequences potentially far more harmful than today's "green-house effect." This accumulation of oxygen caused extinction of most existing life forms, defenseless against oxidation-mediated toxicity. Eventually new life forms emerged and flourished in the new environment. This aerobic life possessed effective antioxidant mechanisms and even began exploiting oxygen and its derivates—so-called reactive oxygen species (ROS)—for production of energy and signal transduction.

One pathway that harnessed the potent reactivity of

ROS as second messengers for signal transduction is the TNF-R1 pathway—critical for controlling inflammation, immunity, cell death, and proliferation (Wajant et al., 2003). This pathway also plays a central role in cancer and chronic inflammation and has attracted major biomedical interest for the last 100 years. Like other so-called "death receptors," TNF-R1 is hardwired into the machinery of programmed cell death (PCD) (Wajant et al., 2003). Yet, despite this potential for killing cells, engagement of TNF-R1 by its ligand, TNFα, does not usually cause death, owed to potent activation of NF-κB transcription factors (Wajant et al., 2003). Several laboratories have now shown that this protective activity of NF-κB against TNFα-induced killing involves suppressing accumulation of ROS and sustained activation of the JNK MAPK cascade (De Smaele et al., 2001; Tang et al., 2001; Sakon et al., 2003; Pham et al., 2004) (Figure 1). These antioxidant and prosurvival actions of NF-κB are mediated in part by upregulation of the iron binding protein, Ferritin Heavy Chain, and the ROS scavenger, Mn^{2+} Superoxide Dismutase (Mn-SOD) (Pham et al., 2004; Kamata et al., 2005). ROS and JNK have both been found to play obligatory roles in TNF-R1-induced killing, and indeed, their activities appear to be linked because ROS-triggered cytotoxicity depends on downstream activation of the JNK pathway (Pham et al., 2004; Kamata et al., 2005). The precise mechanism(s) by which ROS promotes activation of JNK, however, has remained elusive.

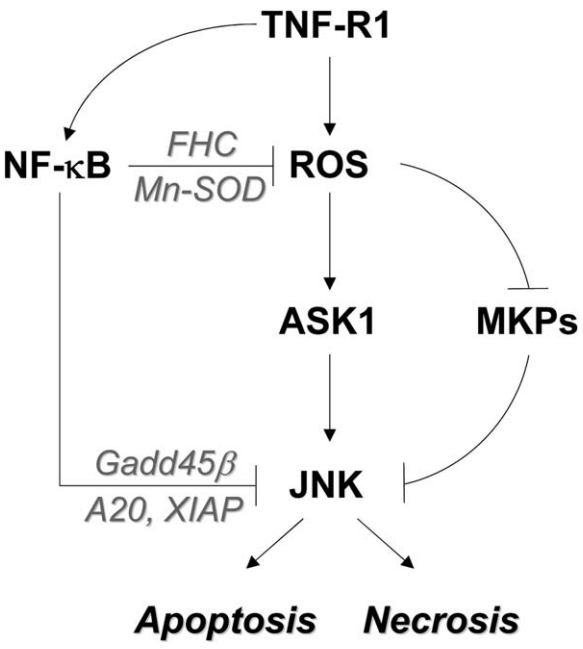

Figure 1. Death and Survival Signaling Pathways Downstream of TNF-R1

Ligand engagement of TNF-R1 initiates accumulation of ROS, which promotes cell death through activation of the JNK MAPK cascade. This occurs via at least two mechanisms: inactivation of MKPs and activation of ASK1. TNFα-induced sustained activation of JNK signaling can then mediate both the apoptotic and caspase-independent necrotic pathways of PCD. Death signaling triggered by ROS-mediated activation of JNK is antagonized by NF-κB, which upregulates expression of protective genes such as FHC and Mn-SOD (suppressing ROS), and Gadd45β, A20, and XIAP (blocking the JNK pathway directly).

A new study by Karin and colleagues (Kamata et al., 2005) now provides one such mechanism. The study identifies JNK phosphatases of the MKP group as critical molecular targets of ROS in TNFα-induced PCD. ROS-mediated inactivation of MKPs—key effectors for extinguishing MAPK activity—seems to involve oxidation of a critical cysteine residue in their catalytic domain. ROS-dependent loss of MKP function then leads to persistent activation of JNK by TNFα, triggering mitochondrial release of cytochrome *c* and, ultimately, PCD via necrosis and apoptosis (Figure 1).

The study provides important new insights into the basis of ROS-mediated activation of proapoptotic JNK signaling and opens new avenues for investigation (see below). Some cautionary notes, however, seem appropriate. First, the proposed model is supported mainly by data obtained with pharmacological and dominant-negative inhibitors and, so, requires genetic validation with knockout systems. Additionally, it is worth noting that inactivation of MKPs is unlikely to be the sole mechanism by which ROS promote JNK signaling. Previous knockout studies suggest that ROS also activate ASK1/MEKK5, a MAPKKK needed for sustained JNK induction and PCD downstream of TNF-R1 (Matsuzawa and Ichijo, 2005). Therefore, ROS might influence JNK activation by controlling both activating kinases and inhibitory phosphatases (Figure 1), the relative importance of which may depend on biological context.

Some controversial issues also await resolution. One pertains to whether NF-κB also blunts activity of p38—another MAPK attenuated by MKPs—as suggested by this study. Other groups have reported, in fact, that the inhibitory action of NF-κB is specific to the JNK MAPK cascade (Tang et al., 2001; Reuther-Madrid et al., 2002). There is also discrepancy in published studies regarding the effects of NF-κB on JNK activation by IL-1β-R (Tang et al., 2001; Sakon et al., 2003; Kamata et al., 2005), which does not promote accumulation of ROS. Resolutions to these issues will help establish which downstream targets of NF-κB are most relevant to NF-κB-mediated cytoprotection in specific contexts.

Notably, the study highlights important challenges for the future. Foremost, the primary source(s) of signal-transducing ROS downstream of TNF-R1 still needs to be identified. It is often assumed that TNFα-induced ROS originate in mitochondria. However, prior studies (including the current one by Kamata et al.) have measured ROS production at relatively late times (i.e., hours), and so, these measurements are likely complicated by the oxidative burst that follows mitochondrial outer membrane depolarization, a sign that cells may have already committed to die. Thus, whether mitochondrial ROS are a cause or secondary consequence of cell death remains unclear. Indeed, the observations that ROS are not induced by IL-1β (Kamata et al., 2005) and that their induction by TNFα is blocked by ablation of JNK1/2 (Ventura et al., 2004) could be simply explained by lack of cell death. The notion of a primarily mitochondrial origin of TNF-R1-stimulated ROS is also

challenged by the weak protective activity of mitochondrial Mn-SOD (Sakon et al., 2003; Pham et al., 2004). Putative extramitochondrial sources of TNF-R1-induced ROS have in fact been identified. Ultimately, identifying the origin of ROS will require employing genetic tools and more sophisticated methods for detecting early ROS and discriminating individual species.

Finally, there is evidence to suggest that the coupling of ROS and JNK signaling downstream of TNF-Rs is bidirectional. It has been proposed that in the TNF-R1-triggered pathway for necrosis, ROS lie downstream (rather than upstream) of JNK (Ventura et al., 2004). Thus, the molecular ordering of JNK and ROS signaling might differ depending upon the type of PCD response initiated by TNF-Rs. Indeed, this represents another important issue for future investigation.

The actual outcome of TNF-R stimulation depends upon the biological context and tissue in which this stimulation occurs. Undoubtedly, major future challenges include determining the precise mechanisms by which ROS promote JNK activation and PCD and assessing which target genes are most relevant to the antioxidant activity of NF-κB in specific tissues and contexts. The use of conditional knockout models will be key for addressing these issues. Because the NF-κB-mediated attenuation of TNFα-induced killing plays a crucial role in human diseases, gaining understanding of how ROS trigger PCD and how NF-κB promotes survival might enable development of entirely novel approaches to treatment of these diseases, one that is effective and yet lacks the serious immunosuppressive side effects of general NF-κB blockers.

The study by Kamata et al. provides an exemplary illustration of this concept. It shows that in the liver, ROS-mediated activation of JNK signaling plays a selective role in TNF-R-mediated hepatic injury induced by concanavalin-A, but not in regeneration postpartial hepatectomy, albeit both processes are governed by integration of activities of TNF-Rs, JNK, and NF-κB. Identifying the mechanisms responsible for ROS-mediated JNK induction and NF-κB-dependent protection in patho-physiological contexts such as these represents a major challenge yet holds great promise of yielding the key for a new type of approach to therapy.

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